IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No.:	**DEX-0087**
Inventors:	**Recipon et al.**
Serial No.	**09/705,500**
Filing Date:	**November 3, 2000**
Examiner:	**Canella, Karen A.**
Group Art Unit:	**1642**
Title:	**A Novel Method of Diagnosing, Monitoring, Staging, Imaging and Treating Cancer**

Declaration under Rule § 1.131

We, Herve Recipon, Roberto A. Macina, Sei-Yu Chen and Yongming Sun, hereby declare:

1. We are co-inventors of the above-referenced patent application.

2. As the co-inventors of the above-referenced patent application, we are familiar with the teachings of the above-referenced patent application.

3. The use of Lng108, also known as fy108, as a cancer diagnostic was conceived and reduced to practice in this country at diaDexus, Inc., prior to October 27, 1999 which was located at that time in Santa Clara, California, USA.

4. Specifically, Lng108 relative expression was determined in accordance with our standard Quantitative Polymerase Chain Reaction (QPCR) protocol and outlined in the above-referenced patent application at page 20, line 16 through page 21, line 13; and page 17, line 12 through page 18, line 9 of priority application U.S. Provisional No. 60/163,144, filed on November 4, 1999. These experiments measuring relative levels of Lng108 in cancerous, normal-adjacent, and normal tissues using Polymerase Chain Reaction in real time were performed prior to October 27, 1999.

Real-Time quantitative PCR with fluorescent Taqman probes is a quantitation detection system utilizing the 5'-3' nuclease activity of Taq DNA polymerase. The method uses an internal fluorescent oligonucleotide probe (Taqman) labeled with a 5' reporter dye and a downstream, 3' quencher dye. During PCR, the 5'-3' nuclease activity of Taq DNA polymerase releases the reporter, whose fluorescence can then be detected by the laser detector of the Model 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA, USA).

Amplification of an endogenous control was used to standardize the amount of sample RNA added to the reaction and normalize the Reverse Transcriptase (RT) efficiency. Either cyclophilin, glyceraldehyde-3-phosphate dehydrogenase (GADPH) or 18S ribosomal RNA (rRNA) was used as this endogenous control. To calculate relative quantitation between all samples studied, the target RNA levels for one sample were used as the basis for comparative results (calibrator). Quantitation relative to the calibrator can be obtained using the standard curve method or the comparative method (User Bulletin #2: ABI PRISM 7700 Sequence Detection System).

The tissue distribution, and the level of the target gene for every example in normal and cancer tissue were determined. Total RNA was extracted from normal tissues, cancer tissues, and from cancers and the corresponding matched adjacent tissues. Subsequently, first strand cDNA was prepared with reverse transcriptase and the polymerase chain reaction was done using primers and Taqman probe specific to each target gene. The results are analyzed using the ABI PRISM 7700 Sequence Detector. The absolute numbers are relative levels of expression of the target gene in a particular tissue compared to the calibrator tissue.

5. Attached are copies of laboratory notebook pages 0034-124, 0034-168 and 0049-048 containing QPCR experimental procedures and results fro Lng108, also known as fy108. The actual dates upon which these experiments were performed in this country at diaDexus, Inc., prior to October 27, 1999 which was located at that time in Santa Clara, California, USA, have been redacted from the attached copies. These experiments, all performed prior to October 27, 1999, demonstrated the use of Lng108 as a diagnostic marker for cancer.

We further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Hervé Recipon — Date 09/07/2004

Roberto A. Macina — Date

Sei-Yu Chen — Date

Yongming Sun — Date

We further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Herve Recipon — Date

Roberto A. Macina — Date 9/7/04

Sei-Yu Chen — Date 8-25-04

Yongming Sun — Date

We further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Herve Recipon — Date

Roberto A. Macina — Date

Sei-Yu Chen — Date

Yongming Sun — August 25, 2004 Date

CONTINUED FROM PAGE 123

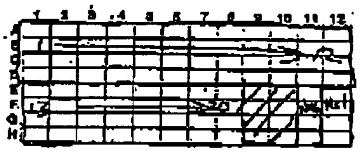
fy108 : N=20

"N=20" PCR Assay Worksheet

PCR Target: fy108 | Name:
Tissue Load: | Date:
Endogenous Control: | Expt:
Target Primers: | Cycler:
Dose of Calibrators: | (N=12 Panel Lot Number)

Master Mix

		TARGET & CONTROL	
	Water	4.85	[illegible]
2x	Master Mix	10.0	[illegible]
100uM	forward	0.05	3.0
100uM	reverse	0.05	3.0
[illegible]	probe	0.05	3.0
		10	
		10 uL cDNA	



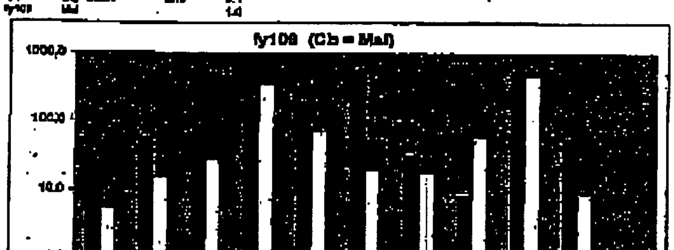
Tissues (paste report below):

Matched Tissue Query

[illegible]

Target	Tissue	CAN	NAT
fy108	Lng [illegible]	5.0	[illegible]
fy108	Lng [illegible]	[illegible]	[illegible]
fy108	Lng [illegible]	[illegible]	28.1
fy108	Lng [illegible]	[illegible]	[illegible]
fy108	Lng LC71	82.1	71.3
fy108	Lng [illegible]	[illegible]	[illegible]
fy108	Lng [illegible]	32.9	[illegible]
fy108	Lng [illegible]	8.7	[illegible]
fy108	Lng [illegible]	[illegible]	[illegible]
fy108	Lng [illegible]	67.9	8.1
fy108	Mal		1.0

paired [illegible] (CAN vs NAT)
p-value = 0.4740



	Lng [illegible]	Lng [illegible]	Lng [illegible]	Lng [illegible]	Lng LC71	Lng [illegible]	Lng [illegible]	Lng [illegible]	Lng [illegible]	Lng [illegible]	Mal
CAN	5.0	[illegible]	[illegible]	[illegible]	82.1	[illegible]	32.9	8.7	[illegible]	67.9	
NAT	[illegible]	[illegible]	[illegible]	[illegible]	71.3	[illegible]	[illegible]	[illegible]	[illegible]	8.1	1.0

END

CONTINUED ON PAGE 125

SCIENTIST SIGNATURE: DATE:
WITNESS SIGNATURE: DATE:

CONTINUED FROM PAGE 167 Lng108: N=20 x2

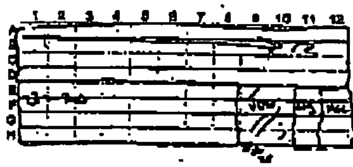
"N=20" PCR Assay Worksheet

PCR Target: Lng108
Tissue Load: Lung
Endogenous Control: Actin (Lot # ...)
Target Primers: F/R (Lot: Yellow)
Date of Calibrators: Mkt, Lng

Name: [illegible]
Date: [illegible]
Expt: [illegible]
Cycler: [illegible]
Nel12 Panel Lot Number: [illegible]

Master Mix

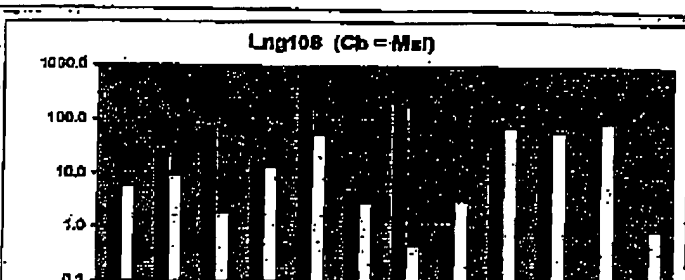
		TARGET & CONTROL	
	Water	4.85 (x50)	245
2X	Master Mix	15.0	750.0
10uM	forward	0.06	3.0
100uM	reverse	0.06	3.0
20uM	probe	0.06	3.0
		20	
		+ 10 UL cDNA	



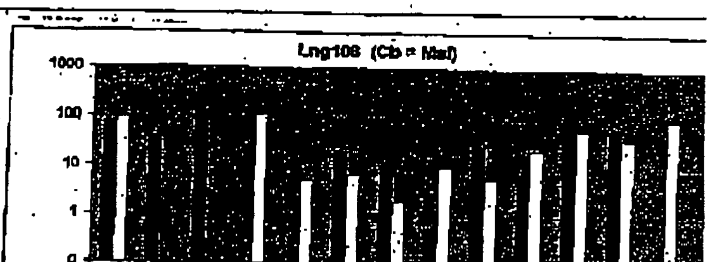
Tissues (see also report below):

Matched Tissue Query

[illegible]



	Bld 46XK	Cln DZXR	Cln TX67	End 12XA	Kid 10XXD	Liv 15KA	Lng 47XQ	Lng 75XC	Lng 5Q48	Lng SD58	Lng	Mal
CAN	3.4	29	78	18	641	6	204	1	31	dq		
NAT/NRM	5.6	10	2	15	55	3	0.5	3	70	65	95	1



	Mam 12X	Ovr 1000Q	Ovr 1028	Ovr 18QA	Ovr 2061	Pan 77X	Pro 12B	Pro 12XQ	Bld H59	Sto AC44	Utr 141XO	Utr ZXXU	Lng
CAN	60	40	106		[illegible]	21	11	0.5	28	6	110	64	
NAT/NRM	97			116	5	7	7	10	8	29	65	41	108

END

CONTINUED ON PAGE 169

SCIENTIST SIGNATURE: CP
DATE:
WITNESS SIGNATURE:
DATE:

CONTINUED FROM PAGE 47 "N=20" fy108

Objective: to evaluate lung lead fy108 with additional 40 can/N matched samples.

Protocol:

20 "N=60" PCR Assay Worksheet

PCR Target: fy108
Tissue Lead: Lung
Endogenous Control: ATPase (Lot) yellow
Target Primers: fy108 (Lot) yellow
Desc of Calibrators: Muscle

Name: Laura A
Date:
Expt: 049 p048A & B
Cycler:

Master Mix

Plate ① 049 p048A

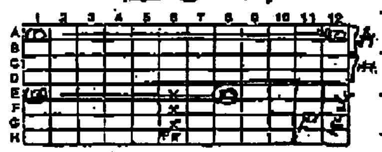


Plate ② 049 p048B
- same set-up as plate ①, but different tissues.
*- no template added to wells E6 & H6 on plate ①

Tissues (paste report below):

Matched Tissue Query

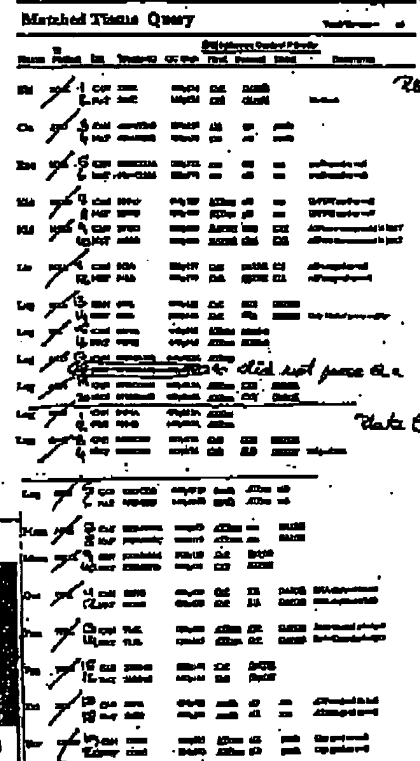


Plate ① 049 p048A
Plate ② 049 p048B
did not pass QC

Plate ①

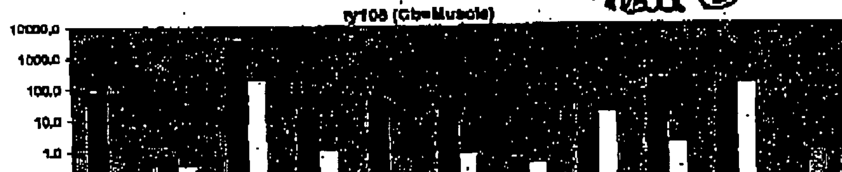
fy108 (Cb=Muscle)

	Bld 32XK	Cln AS67	End 28XA	Kid 106XD	Kid 107XD	Liv 94XA	Lng 60XL	Lng 60X	Lng AC13	Lng AC88	Msd
■ CAN	17.5	28.4	49.2	16.6	1992	30.0	26.9	12.7	18.3	60.2	
□ NAT	4.6	0.1	35.4	62.2	81.0	1.6	5.3	7.3		111.	1.0

Data normalized to ATPase

Plate ②

fy108 (Cb=Muscle)



	Lng BR94	Lng C20X	Lng 9C82	Mam A06X	Mam B011X	Ovr 103X	Pan 71XL	Pro 20XB	Tst 39X	Utr 135XD	Msd
■ CAN	65.0	0.7	3821.	20.9	46.7	44.2	0.5	2.0	164.6	88.0	
□ NAT	NEG	0.4	2163	1.1	0.2	0.6	0.4	15.0	1.6	136.9	1.0

* Exp. 049 p048A

	Baseline	Threshold
fy108	3 to 21	0.03
ATPase	3 to 14	0.02

* Exp. 049 p048B

	Baseline	Threshold
fy108	3 to 21	0.02
ATPase	3 to 14	0.03

CONTINUED ON PAGE 49

SCIENTIST SIGNATURE: Laura A DATE:
WITNESS SIGNATURE: S. Ch DATE: